Prenatal Diagnosis (and a bit of Screening) in the 21st Century

Dr Emma Parry
Clinical Director New Zealand Maternal Fetal Medicine Network (NZMFMN)
Mrs A B

• \( G_1 P_0 \)
• 28 weeks pregnant
• Severe fetal growth restriction with minimal liquor
• Placental cause or possible aneuploidy
Mrs CD

- $G_5P_4$
- Late booker at 28 weeks gestation
- Multiple Fetal Anomalies on USS
- Major cardiac, duct dependant
- Omphalocele
- Scoliosis
- Amniocentesis
- CVS
- Ultrasound
- Current genetic testing
- Array technology
- NIPT
- PGD
- Ethical issues
Amniocentesis

- Prochovnik, Von Schatz and Lambl in 1877
- Hinkel 1919 for polyhydramnios
- Liley 1961 to guide IUTs
• Fuchs and Riis in 1956
  – Genetic analysis of amniotic fluid
  – Fetal sex using Barr body
• Steele and Bregg in 1966
  – Cultured amniotic cells suitable for karyotype
• Nader in 1968
  – One of first diagnoses Trisomy 21
ROLE OF AMNIOCENTESIS IN THE INTRAUTERINE DETECTION OF GENETIC DISORDERS

Henry I. Nadler, M.D., and Albert B. Gerbie, M.D.

Abstract One hundred and sixty-two transabdominal amniocenteses were performed between the thirteenth and eighteenth weeks of fetal gestation as part of the management of 155 “high-risk” pregnancies. Successful cultivation of amniotic-fluid cells led to the intrauterine detection of Down’s syndrome (10 cases), Pompe’s disease (one case), lysosomal acid phosphatase deficiency (one case) and metachromatic leukodystrophy (one case). The risk of this procedure is low since neither fetal nor maternal complications were demonstrated in this series of patients. Cultivation of amniotic-fluid cells obtained by transabdominal amniocentesis early in the second trimester of pregnancy provides a method that enables parents at “high risk” for having offspring with certain serious genetic disorders to have children without risk of such a defect.

AMNIOCENTESIS has been used as a diagnostic aid since the early 1930’s. Since the demonstration of its value in the management of Rh isoimmunization, the technic of transabdominal amniocentesis has gained widespread acceptance. This procedure has been performed over 10,000 times after the twentieth week of pregnancy and fetal morbidity or mortality reported in less than 1 per cent of cases. In adverse effects that have been reported fetal mortality appears to be greater than maternal, with fetal deaths reported due to abruptio placentae, amnionitis and fetal hemorrhage. Puncture of the fetus has been reported. The maternal morbidity includes amnionitis, maternal hemorrhage, abdominal pain and peritonitis. More recently transabdominal amniocentesis has been performed early in the second trimester of pregnancy. In most cases, amniocentesis has been performed immediately before pregnancy was interrupted, making it difficult if not impossible to define the risks to either fetus or mother accurately. Transvaginal amniocentesis has been shown to carry an appreciable risk of spontaneous abortion when performed early in pregnancy.

During the past few years, sex-chromatin analysis
• USS guidance developed in 1970s with ‘X marks the spot’

• In 1980’s freehand single operator approach
Current practice

- 15+0 / 40
- Membranes sealed
- Anti-D if Rhesus negative
- Results timing dependant on testing performed in the laboratory

- Risk of miscarriage
- Risk of PPROM
Mrs A B

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- What now?
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Mrs CD

- Multi disciplinary meeting
- Delivery at a centre with Paediatric Surgery (Auckland, Hamilton, Wellington, Christchurch)
- Cardiac duct dependant and needs delivery at Paediatric Cardiac centre: Starship
- Parents want everything done
- Should we?
CVS

- 1968 TV direct vision CVS by Mohr in Scandinavia
- 1980 Kazy in Russia first USS guided TA
- 1984 Smidt-Jensen and Hahnemann
Current practice

• 11+0 to 13+6 / 40
• Placenta accessible
• Anti-D if Rhesus negative
• Results timing dependant on testing performed in the laboratory

• Risk of miscarriage
• Risk of placental mocaiaisism
Mrs A B

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Ultrasound

- Imaging quality improving exponentially
- 2 X screening/diagnostic scans
  - 12/40 gestation
  - 18-20/40 gestation
- Part of combined screening
- Structural anomalies with no genetic abnormality (or is there?..)
Current Genetic testing

- G banding Karyotype
- FISH
- QF-PCR
- BoBs
Microarrays

- 3-4 Mb

- Single base change

Sequence variation:
- Single nucleotide
  - Base change – substitution – point mutation
  - Insertion-deletions (‘indels’)
  - SNPs – tagSNPs
- 2 bp to 1,000 bp
  - Microsatellites, minisatellites
  - Indels
  - Inversions
  - Di-, tri-, tetranucleotide repeats
  - VNTRs
- 1 kb to submicroscopic
  - Copy number variants (CNVs)
  - Segmental duplications
  - Inversions, translocations
  - CNV regions (CNVRs)
  - Microdeletions, microduplications

Structural variation:
- Microscopic to subchromosomal
  - Segmental aneusomy
  - Chromosomal deletions – losses
  - Chromosomal insertions – gains
  - Chromosomal inversions
  - Intrachromosomal translocations
  - Chromosomal abnormality
  - Heteromorphisms
  - Fragile sites
- Whole chromosomal to whole genome
  - Interchromosomal translocations
  - Ring chromosomes, isochromosomes
  - Marker chromosomes
  - Aneuploidy
  - Aneusomy

Slide courtesy of Trent Burgess GHSV
G-bandig karyotype

- Cell culture (7-14 days)
- Arrested in metaphase
- Scientist examines under microscope
G-bandning karyotype

Advantages

• Complete chromosome analysis
• Moderate resolution (5Mb)
• Can detect rearrangements

Disadvantages

• Length of time for result
• Labour intensive
• Resolution not high enough for many genetic disorders
FISH (Fluorescent In Situ Hybridisation)

- Probe with Fluorescent marker applied to cells
- Probe attaches to proscribed areas
- Reviewed under microscope by scientist and number and colour of fluorescing markers counted
- Wide range of Probes available; Chromosome 13, 18, 21, 22q11.2 etc
QF-PCR

- Small sections of DNA ‘cut up’
- Amplified via Polymerase Chain Reaction
- Primers (fluorescent) used for each section different label and size
- Amount of each section measured automatically
- Result as a graph with amounts
BACs-On-Beads

- Probes immobilised onto Fluorescently labelled beads
- Up to 100 beads detecting different sequences
- Once sample applied to beads, laser reader
- Amount of target bound to each bead type compared to reference
- In prenatal setting standard chromosomes plus 9 microdeletions
## Targeted Approach

<table>
<thead>
<tr>
<th></th>
<th>FISH</th>
<th>QF-PCR</th>
<th>BoBs</th>
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<tbody>
<tr>
<td><strong>Method</strong></td>
<td>Human reads in situ probe</td>
<td>Automated report of relative DNA amounts</td>
<td>Automated report of relative DNA amounts</td>
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<tr>
<td><strong>Advantage</strong></td>
<td></td>
<td>Large volumes easy</td>
<td>Large volumes easy</td>
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<td><strong>Cost?</strong></td>
<td>$$$</td>
<td>$$</td>
<td>$</td>
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<td></td>
<td>Wide range of probes</td>
<td></td>
<td>Can test up to 100 probes at once</td>
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![New Zealand Maternal Fetal Medicine Network](image)
Array Technology

- Array CGH (Comparative Genomic Hybridisation)
- High resolution (200kb)
- Automated
- Cannot detect balanced rearrangements
- Established use in paediatrics
  - 15% vs 3% diagnosis
- Knowledge and registries increasing
Array CGH: The Complete Process

Steps 1-3 Patient and control DNA are labeled with fluorescent dyes and applied to the microarray.

Step 4 Patient and control DNA compete to attach, or hybridize, to the microarray.

Step 5 The microarray scanner measures the fluorescent signals.

Step 6 Computer software analyzes the data and generates a plot.
• Antenatal use
• Different decision making pathway
  – Platform: targeted versus full
  – Variation of Unknown significance
  – Counselling
  – Paternity..
• Abnormal Result ? What now
• Improvement over time
Worldwide experience

- 18/1075 (1.7%) significant aberration undetected by conventional testing (US)

Canada        Houston
New Zealand experience

- Introduced with ongoing audit as collaboration between Genetic Health Service NZ and NZMFMN
- Abnormal sonogram
  - 2 abnormalities (IUGR incl)
  - G banded complex rearrangement
  - Family History Chromosomal rearrangement
- Pretest and post test counselling
New Zealand experience

- Started 2014
- 26 tests performed
- 16 Normal
- 9 Abnormal
- 7 VoUnknown
- 1 VoUncertain
- 1 Pathogenic
NIPT

• Screening not Diagnosis
• BUT cannot be ignored by Cytogenetics or Obstetrics!
Current screening

- Ultrasound
- Biochemical markers
- Maternal characteristics
- Range of risks
Mrs A B

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NIPT


• The fetus/placenta is genetically distinct from the mother

• Detection of Y-chromosome-specific DNA using PCR

• Primary source is trophoblast not the fetus
Cell-free fetal DNA in maternal blood

- Detectable from 5 weeks
- Concentration rises with gestation
- Proportion of fetal DNA vs total (maternal + fetal) DNA in maternal plasma is 10-20% ("fetal fraction")
- Cff DNA is cleared rapidly after delivery
Technical challenges of NIPT for aneuploidy

- DNA sequences from Chromosome 21 arise from both maternal and fetal sources
- Determining fetal chromosome dosage per cell without presence of fetal cells
- High background maternal DNA

Need high precision mapping and counting of DNA fragments to determine if there is more than expected from Chr 21
What is ‘next generation sequencing’?

• Sequencing = determining base sequence in a segment of DNA
• Sanger sequencing
• NGS based on a similar approach of synthesizing DNA but ‘massively parallel’
NIPT for fetal aneuploidy

Cell-free DNA in placenta → Cell-free DNA in maternal plasma

Maternal DNA → Red blood cell → Fetal DNA → Aneuploidies

Massively parallel sequencing of total DNA present in maternal plasma → Alignment of sequencing reads to human genome sequence and determination of relative chromosome representation → Detection of aneuploidy e.g. trisomy 21

New Zealand Maternal Fetal Medicine Network
Factors that influence accuracy of NIPT

- Sequencing depth
  - Coverage: average number of sequencing reads
- GC base content
  - More stable bonding than AT
  - Positive bias chromosomes with more i.e. 21, 18 and X
- Fetal Fraction
  - Median FF at 11-13 weeks 10%
  - Minimum 4% needed for most NIPT
NIPT

- Rhesus blood type, Autosomal Dominant conditions
- Massive Parallel Sequencing (MPS)
NIPT in high risk population

• Industry-sponsored trials with academic partners
• Studied high risk women undergoing invasive testing
  – Maternal age
  – Hx of previous aneuploidy pregnancy
  – High risk screening result
  – Abnormality on USS
• Wide range GA >/= 10/40
• Comparisons of NIPT with results in karyotype:
  – Trisomy 21
    • Sensitivity >99.5%
    • Specificity >99.5%
NIPT in a low risk population

DNA Sequencing versus Standard Prenatal Aneuploidy Screening

Diana W. Bianchi, M.D., R. Lamar Parker, M.D., Jeffrey Wentworth, M.D., Rajeevi Madankumar, M.D., Craig Saffer, M.D., Anita F. Das, Ph.D., Joseph A. Craig, M.D., Darya I. Chudova, Ph.D., Patricia L. Devers, M.S., C.G.C., Keith W. Jones, Ph.D., Kelly Oliver, B.S., Richard P. Rava, Ph.D., and Amy J. Sehnert, M.D., for the CARE Study Group

ABSTRACT

BACKGROUND
In high-risk pregnant women, noninvasive prenatal testing with the use of massively parallel sequencing of maternal plasma cell-free DNA (cfDNA testing) accurately detects fetal autosomal aneuploidy. Its performance in low-risk women is unclear.

METHODS
At 21 centers in the United States, we collected blood samples from women with singleton pregnancies who were undergoing standard aneuploidy screening (serum biochemical screening or noninvasive prenatal test) before 14 weeks of gestation. We performed...
Non-invasive prenatal testing for fetal chromosomal abnormalities by low-coverage whole-genome sequencing of maternal plasma DNA: review of 1982 consecutive cases in a single center


*Fetal Medicine Centre, Paramount Medical Centre, Hong Kong, China; †Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; ‡Clinical Laboratory of BGI Health, Shenzhen, China; ¶Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong, China; §§Shenzhen Research Institute, The Chinese University of Hong Kong, China

KEYWORDS: aneuploidy; chromosomal deletions; chromosomal duplications; fetal DNA; next-generation sequencing; NIPT; sex chromosomal abnormality; whole genome

ABSTRACT

Objective To review the performance of non-invasive prenatal testing (NIPT) in a real-life setting.

The non-invasive prenatal testing (NIPT) of plasma DNA has been widely adopted in clinical practice. In this study, we aimed to review the performance of NIPT in a single center.

Methods A total of 1982 singleton pregnancies were offered NIPT from April 2010 to June 2013. The prevalence of abnormal chromosomes in NIPT-negative cases was ascertained in 1645 (85.15%).

Results Three chromosomal abnormalities were not detected by NIPT, including two fetal anomalies and one maternal anomaly.

Conclusion NIPT is an effective screening tool for fetal chromosomal abnormalities, but NIPT-negative cases should be followed up with standard prenatal diagnostic methods.
• Blood taken at 12 weeks
• 8 day turnaround
• Whole genome approach
• Positive overall
  – 38/1959 = 1.94% or 1 in 52
• Trisomy 13,18,21
  – 19/1959 = 0.97% or 1 in 103
• ‘Failed’ test, repeat needed 1.16%
• 1 false positive with Trisomy 9
Drawbacks

- Concern amongst MFM's re conflict in studies. All company driven
- Audits needed of implementation
- Expensive
- Turnaround time
- Failures and low free fetal fraction
- Obesity
- Multiple and vanishing twin
- Screening and invasive test to confirm
Potential diagnostic consequences of applying non-invasive prenatal testing: population-based study from a country with existing first-trimester screening

O. B. PETERSEN*, I. VOGEL†#, C. EKELUND‡, J. HYETT§, A. TABOR †, the Danish Fetal Medicine Study Group and the Danish Clinical Genetics Study Group

*Fetal Medicine Unit, Department of Obstetrics, Aarhus University Hospital, Aarhus, Denmark; †Department of Clinical Genetics, Aarhus University Hospital, Aarhus, Denmark; ‡Fetal Medicine Unit, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark; §Department of High Risk Obstetrics, Royal Prince Alfred Hospital, Sydney, Australia

KEYWORDS: chromosomal anomaly; free fetal DNA; NIPT; prenatal diagnosis; prenatal screening

ABSTRACT

Conclusions A significant proportion of karyotypic
• cFTS (MSS1) in 193,638 pregnancies
• 10,205 (5.3%) had cytogenetic or molecular analysis
• 1,122 (11.0%) abnormal karyotype
  – 262 (23.4%) would have been missed by NIPT but probably clinically significant
  – >45 years, NT >3.5mm/MSS1 over represented
Mrs A B

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- NIPT Reassuring
Mrs CD

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- Omphalocele
- Scoliosis
- NIPT Trisomy 18
DNA testing fetus leads moms to their own cancer diagnoses

Meredith Knight | March 19, 2013 | Genetic Literacy Project

Last year 800,000 woman used non-invasive prenatal testing to find out if their fetuses had genetic disorders like Down Syndrome. And 26 of them also discovered they had cancer.

Non-invasive prenatal testing (NIPT) analyzes DNA from a blood sample taken from a mom-to-be. Up to 10 percent of the genetic material circulating in a pregnant woman’s blood stream comes from her fetus. Fetal cells pass through the placenta and into a mother’s bloodstream. These genetic tests can identify the non-maternal DNA and check it to make sure it has the right number and quality of chromosomes meaning the baby will be free of genetic disorders.

Tumors also shed their DNA into the bloodstream, although less than a fetus. And cancer cells often have major chromosomal abnormalities that can be identified, just like Down Syndrome, by a NIPT test.

Eunice Lee took an NIPT test at 10 weeks pregnant Virginia Hughes at BuzzFeed reports. Her results indicated she might have cancer:
"I feel like the luckiest person alive," Dr Lee, a San Francisco-based anesthesiologist, said.

She had NIPT, DNA patterns “unusual”

When she was 15 weeks pregnant, she underwent a whole-body MRI scan and a 7 cm tumor in the sigmoid colon was detected.

With laparoscopic resection, the surgeons were able to completely remove the tumor. Postsurgical staging showed that it was T3N0M0 colon cancer.
Brave new world

• NIPT AND Array...

“so can I find out if the baby has blue eyes with this blood test?”
Have we opened Pandora’s box?
PGD

- Common aneuploidies
- Sex selection
- Targeted genetic conditions eg:
  - Metabolic
  - CF
  - Adult onset? Huntingdons
- Choosing normal heterogeneity??
Ethical issues

• What are we trying to achieve with Prenatal screening and diagnosis?
• Rights of the Individual versus society
• Costs to make all diagnoses
• Parents decisions on the information may include termination of pregnancy
  – Poor information versus clarity
• ‘..in countries where prenatal screening is offered as a public health programme, governments ... should adopt an active role to ensure the responsible innovation of prenatal screening on the basis of ethical principles.'
• Crucial elements are
  – the quality of the screening process as a whole (including non-laboratory aspects such as information and counseling),
  – education of professionals,
  – systematic evaluation of all aspects of prenatal screening,
  – development of better evaluation tools in the light of the aim of the practice,
  – accountability to all stakeholders including children born from screened pregnancies and persons living with the conditions targeted in prenatal screening
  – promotion of equity of access’
Accepted Manuscript

SMFM Consult Series #36: Prenatal Aneuploidy Screening using Cell Free DNA

Society for Maternal-Fetal Medicine (SMFM)

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DOI: 10.1016/j.ajog.2015.03.043
Reference: YMOB 10331


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<table>
<thead>
<tr>
<th>No.</th>
<th>Recommendations</th>
<th>Grade</th>
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<tbody>
<tr>
<td>1</td>
<td>Optimal candidates for routine cfDNA aneuploidy screening are women with:</td>
<td>1B.</td>
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<tr>
<td></td>
<td>• Maternal age 35 years or older at delivery</td>
<td>Strong recommendation,</td>
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<td>• Fetal ultrasound finding indicating an increased risk of aneuploidy,</td>
<td>moderate quality</td>
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<td></td>
<td>specifically for trisomies 13, 18 or 21</td>
<td>evidence</td>
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<td></td>
<td>• History of prior pregnancy with a trisomy detectable by cfDNA screening</td>
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<tr>
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<td>(trisomies 13, 18 or 21)</td>
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<td>• Positive screening results for aneuploidy including a first trimester,</td>
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<td>sequential, integrated or quadruple screen</td>
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<td>• Parental balanced Robertsonian translocation with</td>
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<td>increased risk of fetal trisomy 13 or 21.</td>
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<td>2</td>
<td>Routine screening for microdeletions with cfDNA is not recommended.</td>
<td>1B.</td>
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<td>3.</td>
<td>For women who desire comprehensive testing for chromosomal disorders,</td>
<td>1B.</td>
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<td>diagnostic testing should be offered.</td>
<td>Strong recommendation,</td>
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<tr>
<td></td>
<td></td>
<td>moderate quality</td>
</tr>
<tr>
<td></td>
<td></td>
<td>evidence</td>
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<tr>
<td>4</td>
<td>For women undergoing cfDNA aneuploidy screening, MSAFP and/or second</td>
<td>Best practice</td>
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<td>trimester anatomy ultrasound should also be performed.</td>
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<td>5</td>
<td>Formal genetic counseling by MFM subspecialist, geneticist, or genetic</td>
<td>Best practice</td>
</tr>
<tr>
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<td>counselor following a positive cfDNA</td>
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Summary

• Laboratory techniques are evolving at an amazing pace
• Many doctors at the Coalface can’t spell all the assessments
• Doctors being educated by companies
• Colleges are trying to move as quickly as technology with advice..
• Public debate is behind the technology...
• But thanks to social media not far!!
Results for #nipt

Sam Ayres @SamRoseA · 3h
#ESHG and #ASHG highlight in importance of pre test counseling and caution over-expansion of scope with #NIPT nature.com/…

Gustav Karlberg @G_Karlb erg · 4h
Prenatal aneuploidy screening using cell free DNA - The Society for Maternal-Fetal Medicine shar.es/1gmrzRg #NIPT

Captain Sequence @CaptainFuture__ · 13h
Cypher Genomics Broadens Reach into Cancer with Updated Analysis Software Mantis $SQNMQ genomeweb.com/business-news/… #ACMGmtg #NIPT #Liquidbiopsy

Cypher Genomics Broadens Reach into Cancer with Updated Analysis...
The updated Mantis provides researchers a tool for the automated interpretation of cancer-related somatic variants, including variants of unknown significance.
St Georges NHS Trust, UK

• 23rd March 2015
• IONA test (Premaitha) being introduced as low risk screening
• First in the UK
• The test is currently the subject of a patent infringement suit filed by NASDAQ listed Illumina Inc.
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Thank-you

- Professor Peter Stone
- Trent Burgess
- The internet!